World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.008

EFFECT OF DIETARY INDIGENOUS MALAYSIAN CYANOBACTERIA SPECIES, *ARTHROSPIRA GEITLERI* SUPPLEMENTATION ON FEED CONVERSION RATIO AND BLOOD SERUM CHEMISTRY IN LOCAL VILLAGE CHICKEN, *GALLUS DOMESTICUS*

Lokman Shamsudin*[1,2], Syarifah Ab Rashid[1], Azman Nirmal Abdullah[2], Wan Zahari Mohamed[2], Abd Rahman Aziz[2], Mohd Haaziq Saari[2] and Hafis Harres Lokman[1]

[1]Institute of Food Security and Sustainable Agriculture (IFSSA), Universiti Malaysia Kelantan, Locked Bag No 100, 17600 Jeli, Kelantan, Malaysia.
[2]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Jalan Padang Tembak, 16100, Kota Bharu, Kelantan, Malaysia.

***Corresponding Author: Lokman Shamsudin**
Institute of Food Security and Sustainable Agriculture (IFSSA), Universiti Malaysia Kelantan, Locked Bag No 100, 17600 Jeli, Kelantan, Malaysia.

Article Received on 10/01/2019 **Article Revised on 30/01/2019** **Article Accepted on 21/02/2019**

ABSTRACT

The high protein indigenous Cyanobacteria (*Arthrospira geitleri*) meal supplementation was fed to local female chicken, *Gallus domesticus* to ascertain its feed conversion, blood serum chemistry, anti-hyper-cholesterolemic effect and growth performance. Forty healthy female chickens (0.39±0.02 kg) were randomly allotted into one group fed standard diet (SD) and three other groups fed with different strength of *Arthrospira* supplement treatments for 40 days experimental feed trials. The dosage for the three diet groups viz. 1T group was fed *Arthrospira* supplement strength of 1g/0.5kg BW/d, 2T group was fed *Arthrospira* supplement strength of 2g/0.5kg BW/d while 3T group was fed 3g/0.5kg BW/d. The 3T group had elevated significantly (P<0.5) HDL levels by 9.2% (1.65±0.56 mmol.L^{-1}) of its initial value (1.51±0.16 mmol.L^{-1} at day 0); while lowered the total cholesterol (TC) by 20.5% (3.10±0.66 mmol.L^{-1}) of its initial value (3.90±0.46 mmol.L^{-1}) on day 40 respectively during the study period. Furthermore, the blood TG and LDL levels were lowered by 27.7% (initial value of 12.6±0.03 mmol.L^{-1}) and 40% (initial value of 0.30±0.02 mmol.L^{-1}) for the 2T group respectively. This reflects the ability of the *Arthrospira* supplement to function as an anti-hypercholesterolemic effect on the treated chicken. The liver-kidney markers of the experimental feed trials showed that all the treated chicken, viz. GGT appeared to remain stable; however, the blood BUN, creatinine and urea increased by 43%, 12% and 48% for the treated group (3T) respectively of their initial levels all the way throughout the experimental period. Moreover, *Arthrospira* supplementation of the chicken had also elevated significantly (P<0.5) the protein levels by 15% and calcium by 5% of their initial levels for 3T group on day 40 respectively. *Arthrospira* supplementation of the chicken health and growth performance as well as its anti hypercholesterolic effect did not show any significant relatively gradual improvement with increase in the *Arthrospira* strength in term of dosage. There was insignificant differences between all groups in terms of their average final weights (p>0.05). Insignificant differences were observed in feed conversion rate (FCR) and weight gain. However, there were significant differences in terms of feed conversion rates between groups (p<0.05) and the value was better in those treated with *Arthrospira* supplementation ranging from 3.29 to 3.34 when compared to the SD group with an average value of 5.53. It has been shown that the best percentage live weight gain (308.2±29.5 g) and FCR (3.29±0.14) were recorded in *Arthrospira* treated group (3T). This also reflects the ability of the high protein meal supplement to function as an anti-hypercholesterolemic agent in local chickens. It also provided the betterment in the chicken health, blood chemistry and growth performance. The local indigenous *Arthrospira geitleri* effectively reduced the serum lipid levels and simultaneously induced the growth performance in chicken.

KEYWORDS: Cyanobateria, *Arthrospira*, blood serum chemistry, lipoprotein, *Gallus domesticus.*

1. INTRODUCTION

The local village chicken, *Gallus domesticus* is classified as the free ranging chicken or organic chicken and it was known to originate from the cross breed *Gallus gallus* derived from the jungle red chicken (Edjeng Suprijatna, 2008). The important of chicken (poultry) supplementary feeding has been well realized in the modern livestock farming and has been stimulated intensive research on the formulation of cheaper feeds. Many attempts were made to develop cheaper nutritive diets with local high protein bacterial and plant source as the principal sources of protein. These ingredients are rich in protein and are easily available at low cost. The diets developed can be tested by comparing their performance with a standard commercial diet fed to poultry. Hitherto, chicken (broiler), Japanes quail and pigeon are the most common avian species tested for atherosclerosis (Shih et al., 1983).

Protein is the main constituent of the poultry body; as such, a generous dietary supply is needed for rapid growth. Furthermore, protein is more expensive than carbohydrate or fat therefore the amount of protein in the diet should be limited to that which is needed for growth and tissue repair while the energy should come from the cheaper sources. As for the protein level in diets, poultry required a high percentage of protein in their diet than do than other warm blooded animals. For example, the optimum level of protein in practical diets for poultry is 16 to 22% comparable to that of the warm water fish diets (Pond et al.,2005)..

Arthrospira sp. (also known as *Spirulina* sp.) is enriched in protein (70%), essential fatty acid, vitamins, minerals and pigments (carotenoids & chlorophyll); all of these components have been proven to serve as a therapeutic agent for anti-cholesterol (Kim & Kim, 2005), anti-oxidant (El-sabagh et al., 2014) and anti-inflammatory (Muhammad Nazrul et al., 2014). It is important to determine the plasma blood chemistry because the cholesterol-protein package which also known as lipoprotein travels through the blood. Theoretically, the plasma can also be used to evaluate the kidney and liver status by assessing the levels of blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and bilirubin.

Studies have shown that hypercholesterolemia state could contribute significantly to the development of atherosclerosis and coronary heart disease in both human and animal. Hypercholesterolemia is a state of elevated serum cholesterol which results from the overproduction and under-utilization of low density lipoprotein. One of the earliest reports on the reduction of blood serum cholesterol by high plant protein in Cyanobacteria, *Spirulina* was carried on rats by Devi and Venkataranam (1983).

The vast majority of chickens that we ate daily was gravely devastated by adulteration of poultry feeds with unnecessary growth hormones and antibiotics. To replace or supplement the feed with quality nutritive food sources preferably from animal or plant origins rich in protein, minerals and vitamins is of upmost importance. The determination of plasma blood chemistry in terms of the total cholesterol, triglyceride and high density lipoprotein is important to evaluate the health status of the poultry liver and kidney.

In the present study, we carried out an experimental research on the effect of dietary supplementation of the locally (Malaysia) cultured indigenous *Arthrospira geitleri* for its high protein source on the growth, feed conversion and plasma lipoprotein activity in chicken cultured for human consumption**.**

2. MATERIAL AND METHODS

The indigenous local Malaysian strain of the cyanobacteria, *Arthrospira geitleri* used as the chicken supplement is isolated from the pristine alkaline tropical lake, Tasik Dayang Bunting, Langkawi, Kedah, Malaysia; this was grown in modified Luqzi's medium (Lokman Shamsudin *et al*., 2018) at an ambient temperature of 30±2^0 C, alkaline pH (8.5 to 9.0) and under cool while fluorescent source (30 µmol photon $m^{-2}s^{-3}$). The Cyanobacteria is subsequently mass cultured in the 300-L Plug Flow Reactor, filtered, dried and finally powered as supplement feed for the chickens. The microalga which contained high concentration of phytopigments, viz phycocyanin and carotene, can be dried in the form of dried flake mass or powder (Fig.1).

Fig. 1: Confocal Microscopy of i) *Arthrospira geitleri*; ii) *Arthrospira geitleri* in the form of dried flake mass iii) *Arthrospira geitleri* powder rich in phycocyanin & carotene from left to right.

The proximate analysis of crude protein, fiber, lipid, fat, moisture and phytonutrient were carried out in the nutrition laboratory of the Faculty of Veterinary Medicine. Blood chemistry and other related pathophysiological tests were carried out in the path-lab facilities of both the private and the University. The mature fully grown healthy female chicken, *Gallus domesticus* obtained from the local poultry farm, were kept in the animal room controlled at 22±1^{0}C temperature and 55±5% humidity under lighting at intervals of 12 h.

The chickens were divided into 4 groups of 8 individuals (body weight, 0.42±0.02 kg). The healthy chickens were randomly allotted into four groups fed with different treatments for 40 days experimental feed trials, viz. one group fed standard commercial diet (SD control group) and three other groups fed with different diets (1T, 2T and 3T) containing variable strength of high protein indigenous *Arthrospira* supplement. The dosage for the three diet groups viz. 1T group was fed *Arthrospira* supplement strength of 1g/0.5kg BW/d, 2T group was fed *Arthrospira* supplement strength of 2g/0.5kg BW/d while 3T group was fed *Arthrospira* supplement strength of 3g/0.5kg BW/d.

Research animal and experimental set-up.

Forty healthy local chickens, *Gallus domesticus* under experiment were fed twice daily with these diets. Body weight was recorded once a week, and food intake was weighed at every other day during experimental period. The crude protein, ash and moisture contents were determined following AOAC (1975) procedures. The chickens were subjected to intravenous injection of heparin at the dose of 200 U/100g body weight through the jagular vein under anesthesia with Numbutal after fasting for 12h. Plasma total cholesterol, HDL-cholesterol, triacylglycerol, and HDL were determined by enzymatic methods using assay kits (Takayama et al., 1977). Statistical analysis was performed by ANOVA while the significant difference between the different diet groups was done with the t-test.

Laboratory set-up and biochemical pathophysiological tests

For the blood analysis, 3 ml blood was withdrawn from the chicken jagular vein using a needle with the size of 23 G (Terumo, Japan). The blood samples were centrifuged at 5000 rpm for 10 min before being subjected onto automatic analyzer Roche Coba Mira (Thermo Fisher Scientific, USA) and Hitachi 902 Machine for serum cholesterol and kidney-liver analysis specifically total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), high density lipoprotein (HDL), aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), creatinine (Cr), blood urea nitrogen (BUN) and minerals (Ca, P). All blood samples were collected at day 0 (pre-treatment), 20, 30 and 40 (post-treatment).

Statistical analysis

The blood results were determined for statistical significance (p=0.05) using one-way ANOVA by SPSS statistical software (SPSS 16.0 for windows) and Tukey's test was used for pairwise comparison of the mean values.

Ethical guideline

The ethical guidelines on handling the experimental animals were in observance with the standard operational procedure of animal ethics which were verified by the committee of the Faculty of Veterinary Medicine, University Malaysia Kelantan (UMK) and the Kelantan Veterinary Services Department.

3. RESULTS AND DISCUSSION

Chicken growth performance

Table 1 showed the proximate composition of feed materials used in the experiment for feeding local female chickens; it showed that all values of the feed composition changed significantly, especially protein and lipid contents in the experiment diets. The protein content in the treated 1T, 2T and 3T diets is comprised of single, double and triple in the Cyanobacteria, *Arthrospira* concentration respectively as compare to that of the *Arthrospira* content in the SD diet group. The *Arthrospira*-supplemented groups (1T,2T and 3T) contained superior values of the crude protein with values of protein content of 104%, 160% and 216% higher than that of the control group (SD) respectively. The control group (SD) had also lower lipid content by almost half than those the *Arthrospira*-supplemented groups (1T, 2T and 3T).

The present experiment showed that chicken fed with 3g/0.5kg BW/d (3T group) *Arthrospira*- supplementation had significantly increase ($P<0.05$) of the body weight gain as well as lower its FCR. The highest FCR with a value of 5.53±0.21, denoting inefficiency in feed conversion was observed in the standard diet (SD). The increase of weight in 1T, 2T and 3T groups was most likely to be impacted by the higher crude protein contents in these dietary feeds. As shown in Table 1, a value of 104%, 160% and 216% protein in *Arthrospira*-treatment groups managed to augment to 28 - 41% folds of weight gain in the chicken respectively. FCR with values ranging from 3.18 to 3.20 derived from the chicken fed *Arthrospira* were noticeably better than the SD group with a mean value of 5.53. Thus, the combination of dietary feed and *Arthrospira* supplement provides better protein content as well as has given beneficial effect on body weight and FCR. In actuality, FCR can stimulate the body weight gain as well as it served as a good indicator of how proficient a consumable feed can be. Theoritically, it is stated that the lower the FCR, the better the weight gain attained from the feeds.

The present finding was in line and in agreement with those results reported by Kaoud (2012), Kharde et al. (2012), Shanmugapriya & Saravana Babu (2014) and Shanmugapriya et al. (2015). In contrast, other researchers suggested that the *Arthrospira* supplemented diets did not give any significant influence towards the body weight or the FCR on broilers (Mariey et al., 2012; Bonos et al., 2016). Such contradictory results could be due to certain influencing factors such as the dissimilarity of inclusion and quality grades during the feeding trials, the biochemical nutrients in the feed, housing condition and the production systems. The local indigenous *Arthrospira geitleri* contains high protein

(65-70%) comprised those of the essential amino acids, especially leucine, valine and soleucine (Nagaoka et al., 2005: Gershwin & Belay, 2008). It has been reported that the three branched chain amino acids had the ability to increase and enhanced poultry body fat deposition; hence this will increase poultry & bird body weight gain (Peganova & Eder, 2003; Tuttle & Balloun, 1976).

Similar to that of ruminants, chicken rely on the microbial population in the gut to manufacture many of the amino acids and vitamins required for the chicken health performance and production. The quantity of protein in the diet is more important than quality of the protein. As such, health and performance of poultry are influenced by the availability of gut microflora. The complete nutrients in *Arthrospira* sp. such as essential amino acids, unsaturated fatty acid, vitamins, minerals have probably enhanced the gut microflora to exude extracellular enzymes which consequently enhanced the gut microflora to exude extracellular enzymes which increased the gastrointestinal efficiency in animals (Tovar-Ramirez et al.,2002; Gershwin & Belay, 2008). Furthermore, it had been reported that the dietary of 2g/kg of *Arthrospira* sp. had positively affected the ileocecal microbiota by reducing the total bacteria and coliform counts as well as inducing the Stretococci growth rate (Bhowmik et al., 2009). Such occurrence suggested that the *Arthrospira* sp. supplementation had the stimulatory influence on the coccus shaped bacteria which could balance the optimal accumulation of good and harmful pathogenic bacteria. This situation will evitably lead to the betterment of the growth, health and its physiological performance (Bhowmik et al., 2009).

Precisely, it is noted that the higher lipid content in the *Arthrospira* supplemented groups (1T,2T and 3T) as compared to that of the control group (SD) lowered the ash content by almost 29% loss. The protein and lipid are commonly linked to growth performance in term of the body weight gain which is in turn could be relate to feed efficiency such as feed conversion ratio (FCR). In actuality, lipid rich feed is composed of the highly saturated fat which has been suggested to be the agent responsible to trigger hypercholesterolemic event in human (Wardlaw & Snook, 1990; Almendingen et al., 1995; Engel & Thostrup, 2015) as well as in animal (Snowmya & Ananthi, 2011).

Together with protein, high content of lipid is related also to weight gain. It should be noted that all the three feed meal components have different energy component viz. fat having 9 kcal/g while protein and carbohydrate with 4 kcal/g each respectively. Fat has been suggested to be the most efficient fattening factor in weight gain (Swinburn et al., 2004). According to Schwartz et al., (1992) the feed that content higher fat might promote the weight gain because it owns a lower thermic effect compare to carbohydrate or protein.

In constrast, among the many farmed livestock, fish is comparatively the most efficient of animals in converting food energy to body tissue, partly because they require less than 10% of the energy for maintenance required by poultry, birds or mammals of the same size (Gatlin *et al*., 1986; NRC, 1993). Supplemented feed often expressed as a percentage of dry matter, seem to be high in protein content; thus what appear to be a high requirement for protein is really a low requirement for energy. Proteins and lipids in food are important energy sources for many species of commercially raised poultry; however the value of dietary carbohydrates varies especially among species (NRC, 1994). Demands for energy are influenced by physical activity, water temperature, body size and stress. As a comparative study, protein and essential amino acid requirements have been primarily studied in juvenile fish viz. channel catfish declined as the fish approached maturity (Page & Andrews, 1973).

The Cyanobacteria, *Arthrospira* (commonly known as *Spirulina*) can be used as a partial supplementation or complete replacement for protein in livestock feeds and is a cheaper feed ingredient than any other animal origin (Lokman-Shamsudin, 2018). *Spirulina* is composed of 65% protein, 20% carbohydrate, 5% fat, 7% minerals, and 3-6% moisture; this make it a low fat, low calorie and cholesterol free source of protein. This particular indigenous species of *Spirulina* protein has a balanced composition of amino acids with significant concentration of methionine, cysteine, tryptophan and other amino acids almost similar to those of casein; however this depends upon the culture media used. Interestingly, it contains high amounts of phytonutrients, viz polyunsaturated acids PUFAs, phycocyanin, carotene and different type of essential antioxidant plant pigments.

Table 1: The proximate composition of the experimental chicken diets.

Component	(%) SD	1T	2T	3T
Crude protein	11.6±1.37[a]	23.7±1.77[b]	30.2±2.17[c]	36.7±3.11[d]
Crude lipid	4.12±0.87[a]	8.28±0.47[b]	8.25±0.41[b]	8.20±0.57[b]
Crude fiber	3.51±0.31[a]	4.28±0.41[b]	4.31±0.37[c]	4.33±0.24[c]
Moisture	5.83±0.51[a]	5.90±0.31[b]	5.88±0.57[bc]	5.91±0.53[bc]
Ash	5.79±0.34[a]	4.15±0.24[b]	4.14±0.43[bc]	4.12±0.36[bc]

SD standard diet, 1T diet with single strength *Arthrospira*, 2T standard diet with twice strength *Arthrospira*, 3T standard diet with trice strength *Arthrospira*; Different superscripts for the same variable mean implied statistical differences (p<0.05), N number of animals in the group=4.

There were significant different among all groups ($p<0.05$) in term of growth parameters; however, the *Arthrospira* fed meal groups viz. 1T, 2T and 3T had increased their weight gain by 28.8% (280.1±12.2g), 36.6% (298.1±31.3g) and 41.2% (308.3±29.5g) respectively when compared to the value obtained for the SD(218.1±19.1g) respectively (Table 2). The values for the feed convertion ratio (FCR) appear to be better in performance in the treated chicken groups than that of the value obtained from that fed with standard diet. The FCR for the treated chicken groups range from 3.29 to 3.34 which is more favourable than that of the untreated SD standard diet with a FCR value of 5.53. As a comparison to chicken, Kamalaveni et al., (2009) and Salehi-Farsani et al., (2014) recorded that the farmed aquacultured catfish fed high protein content cyanobacteria, *Arthrospira* sp resulted in comparatively better food conversion factor (FCR) than if fed with normal commercial diet. FCR is characterized as the amount of feed given and consumed per unit of weight gain. It is noted that high FCR value in high lipid cholesterol fed the catfish can be related to the bad quality feed, lack of natural resources, poor growth condition and overfeeding (Coche & Muir, 1998). Iwata et al., (1990) reported that high protein cyanobacteria had an inhibitory effect on the elevation in plasma cholesterol, triglyceride, and phospholipid in the fructose induced rats.

Table 2: Growth performance and survival rate of chicken fed with experimental diets for 40 d of treatment with diets (SD, 1T, 2T and 3T).

Growth performance	SM	1T	2T	3T
Initial weight (g)	404.2±12.7^{a}	391.2±13.5^{b}	402.3±17.1^{c}	411.6±16.2^{c}
Final weight (g)	598.2±52.6^{a}	702.3±13.3^{b}	710.7±16.2^{c}	723.9±16.1^{c}
Weight gain (g)	218±19.1^{a}	281.1±12.2^{b}	298.2±31.3^{c}	308.2±29.5^{c}
FCR	5.53±0.21^{a}	3.34±0.13^{b}	3.29±0.14^{c}	3.31±0.16^{d}

SD standard diet, 1T diet with single strength *Arthrospira*, 2T standard diet with twice strength *Arthrospira*, 3T standard diet with trice strength *Arthrospira*; Different superscripts for the same variable mean implied statistical differences ($p<0.05$), N number of animals in the group=4.

Table 3 showed the elevation of serum lipids in term of total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) and high density lipoprotein HDL for a study period of 40 day of treatments imposed on the chickens fed on various diets. Values of plasma cholesterol remained unchanged and stable in control SD group throughout the observation period; however, TC values decreased significantly ($p<0.5$) to 8.7% and 20.5% for 1T and 2T respectively until the end of the experiment. The high *Arthrospira* treated diet (3T) induced a 20.5% (initial value 3.90 ± 0.46 mmol.L^{-1}) fold decrease in plasma cholesterol at the end of 40th day.

Table 3: Values of triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) in chicken for 40 day of treatment with diets (SD, 1T, 2T and 3T).

Time (d)	**10**	**20**	**30**	**40**
TC (mmol.L-^{1})	SD 3.10±0.51^{a}	3.09±0.46^{b}	3.08±0.31^{c}	3.07±0.51^{d}
	1T 3.30±0.46^{a}	3.28±0.41^{b}	3.11±0.22^{c}	3.01±0.36^{d}
	2T 3.50±0.51^{a}	3.21±0.36^{b}	3.01±0.56^{c}	2.90±0.56^{d}
	3T 3.90±0.46^{a}	3.50±0.36^{b}	3.30±0.42^{c}	3.10±0.66^{d}
TG (mmol.L-^{1})	SD 8.98±0.06^{a}	9.02±0.06^{b}	8.99±0.04^{c}	9.05±0.06^{d}
	1T 9.71±0.06^{a}	8.68±0.03^{b}	7.65±0.06^{c}	7.49±0.02^{d}
	2T 12.6±0.03^{a}	11.1±0.06^{b}	10.4±0.07^{c}	9.10±0.06^{d}
	3T13.9±0.06^{a}	12.1±0.08^{b}	11.6±0.06^{c}	9.51±0.03^{d}
LDL(mmol.L-^{1})	SD 0.47±0.03^{a}	0.48±0.06^{b}	0.49±0.07^{c}	0.46±0.06^{d}
	1T 0.32±0.16^{a}	1.28±0.11^{b}	1.21±0.06^{c}	0.24±0.12^{d}
	2T 0.30±0.02^{a}	0.28±0.11^{b}	0.24±0.09^{c}	0.18±0.08^{d}
	3T 0.28±0.06^{a}	0.26±0.06^{b}	0.18±0.06^{c}	0.16±0.06^{d}
HDL(mmol.L-^{1})	SD 1.79±0.14^{a}	1.77±0.16^{b}	1.81±0.19^{c}	1.78±0.16^{d}
	1T 1.39±0.16^{a}	1.40±0.12^{b}	1.43±0.16^{c}	1.94±0.11^{d}
	2T 1.69±0.18^{a}	1.71±0.16^{b}	1.73±0.11^{c}	1.80±0.14^{d}
	3T 1.51±0.16^{a}	1.56±0.11^{b}	1.61±0.16^{c}	1.65±0.16^{d}

SD standard diet, 1T diet with single strength *Arthrospira*, 2T standard diet with twice strength *Arthrospira*, 3T standard diet with trice strength *Arthrospira*; Different superscripts for the same variable mean implied statistical differences ($p<0.05$), N number of animals in the group=4.

The 3T diet group showed a decrease of plasma total cholesterol of 3.10 ± 0.66 mmol.L^{-1} at day 40 from 3.90±0.46 mmol.L^{-1} at day 0; while those of HDL increase slightly to 1.65±0.16 mmol.L^{-1} from 1.51±0.16 mmol.L^{-1} respectively. There was a decrease in the TG value from 13.9±0.06 mmol.L^{-1} to 9.51±0.03 mmol.L^{-1} while those of LDL also decrease tremendously from 0.28±0.06 mmol.L^{-1} to 0.16±0.06 mmol.L^{-1} on day 40. Thus the high protein content in *Arthrospira* supplement was able to reflect as an anti-hyperlipidermic agent which is capable of preventing the high blood cholesterol in chicken. Comparatively, Kato et al., (1984) carried out feeding experiments in rats, and reported that the elevation in total cholesterol (TC), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) and phospholipids in serum caused by cholesterol feeding was reduced when the high cholesterol diet was supplemented with 16% *Spirulina*.

Incidently, this particular local indigenous Malaysian species of *Spirulina* is composed of 68% protein, 20% carbohydrate, 5% fat, 7% minerals, and 3-6% moisture; this make it a low fat, low calorie and cholesterol free source of protein (Lokman-Shamsudin, 2018). It is also rich in essential fatty acids, especially the omega polyunsaturated fatty acids which serve as the main source of the poultry essential fatty acid (Kolanowski *et al.*, 1999; Kris-Etherton *et al*., 2002). *Spirulina* is high in essential polyunsaturated acids PUFA; thus made it a low saturated fat, low calorie and cholesterol free source of protein. Furthermore, *Arthrospira* sp is blessed with the omega PUFA which is responsible in assisting the human physiological processes, growth and development (Simopoulos, 1999), preventing the cognitive decline, dementia, anti cholesterol and anti- inflammatory agents (Calder, 2004; Sinn *et al*., 2012).

Hyperlipidemic events in poultry is detected when the percentage cholesterol range in dietary feed is from 1 to 5% which will develop the cholesterol-induced spontaneous atherosclerosis with lesions found around the gross intimal and aorta (Dauber & Katz, 1942; Dauber, 1944; Horlick & Katz, 1949; Masegi et al., 1993; Orita et al., 1994). However, in broilers the production of lipids, particularly triglycerides are correlated with feed intake as well as the heat stress (Shim et al., 2006). Furthermore, it is reported that broilers required more energy from the dietary feed in order to maintain their homoeothermic condition (Wolfenson et al., 1981; Mckee et al., 1997). Broilers usually neglect their feed in order to minimize the thermogenic effects under the ideal temperature between 20 to 25^0 C and this is related to its efficiency in nutrient absorption, assimilation and utilization (North & Bell, 1990; Daghir, 2008; Tumova & Gous, 2012).

Mariey et al., (2012) reported that 0.2% *Arthrospira* sp. could elevate the serum lipids as well as showing better FCR, increasing the growth performance and the growth performance and lowering the yolk cholesterol. The gamma linolenic acid (GLA), phycocyanin and beta carotene contained in the *Arthrospira* sp have anti-inflammatory with anti-oxidant activities which subsequently affecting the serum elevation in human and animals (Deng & Chow, 2010); this activities will inevitably lessening lipid and protein oxidation. Furthermore, this reflected the action could shield the body from arterial stiffening and atherosclerosis (carter et al., 2000; Carpnter et al., 2003). In actuality, *Arthrospira* sp is involved in the metabolic pathway in reducing cholesterol by binding the cholesterol metabolite bile acid which is available in the liver and later diminished the cholesterol solubility (Drug & Chow, 2010).

The imbalance ratio of n-3 and n-6 PUFA is suggested to be a trigger factor for a few of the chronic inflammatory diseases (Patterson et al., 2012; Strobel et al., 2012). Weaver et al. (2008) and Botta & Ghosh, (2016) reported that feeding chicken with higher n-6 PUFA source, especially from *Arthrospira* sp. is associated with the decrease in n-3 PUFA content; thus, this will inevitably induce the occurrence of hypercholesterolemia in chicken. It has been reported the cyanobacteria, *Arthrospira platensis* has high content of 3-4% n-3 PUFA (Otles & Pire, 2001; Tokusoglu & Unai,2003).

It should be noted that it is necessary to acquire the accurate ratio of n-3 to n-6 PUFA which is able to control or decrease the cholesterol interference physiologically. Interestingly, it has ben reported that the cyanobacteria, *A. platensis* has the ability to reduce the cholesterol in human (Mani et al., 2000) and also in animals such as mice (Belay, 2002), rats (Nagaoka et al., 2005), rabbits (Colla et al., 2008), calves (Heidapour et al., 2011), lambs (El-Sabagh et al., 2014) and non human primate models (Shamekh et al., 2011; Yin et al., 2012). *A. platensis* also is enriched with beneficial pigments such as phycocyanin and carotenoid which are also correlated with the cholesterol reduction (Nagaoka et al., 2005; Colla et al., 2008; Deng & Chow, 2010).

The detailed summarized result of the kidney-liver was shown in Table 4. The liver-kidney markers and related biochemical enzyme indicators in *Arthrospira* fed meal supplement such as BUN, urea, creatinine and uric acid showed significant decrease in their blood values. Furthermore, uric acid which is derived from purine can be found in the feed that is the causing agent for gout in human (Allard, *et al.*, 1994). Table 4 also showed comparatively that blood BUN and urea increased significantly by 55.6% (initial value 2.30±0.26 mg.dL^{-1}) and 50.5% (initial value 0.91±0.11 mmol.L^{-1}) on day 40 for the 2T group respectively. In the case of creatinine and uric acid, their values also showed significant decrease by 15.2% (29.0±2.96 µmol.L^{-1}) and 70.3% (89.0±5.12 µmol.L^{-1}) of their initial values on day 40 for the 2T group respectively.

Table 4: Values of blood urine nitrogen (BUN), urea (U), Creatinine (CR) and uric acid (UA) in chicken for 40 day of treatment with diets (SD, T, 2T and 3T).

Time (d)	10	20	30	40
BUN(mg.dL-[1])	SD 3.90±0.45[a]	3.96±0.36[b]	4.01±0.43[c]	3.97±0.32[c]
	1T 2.00±0.16[a]	1.97±0.16[b]	1.70±0.06[c]	1.10±0.16[d]
	2T 2.30±0.26[a]	1.98±0.16[b]	1.71±0.16[c]	1.02±0.16[d]
	3T 3.08±0.26[a]	2.71±0.26[b]	2.41±0.16[c]	1.75±0.26[d]
Urea(mmol.L-[1])	SD 0.95±0.11[a]	0.96±0.16[b]	1.02±0.11[c]	0.98±0.17[d]
	1T 0.51±0.04[a]	0.48±0.06[b]	0.31±0.02[c]	0.29±0.06[d]
	2T 0.91±0.11[a]	0.89±0.06[b]	0.78±0.06[c]	0.45±0.08[d]
	3T 0.79±0.07[a]	0.61±0.06[b]	0.46±0.06[c]	0.41±0.06[d]
CR(µmol.L-[1])	SD 22.1±22.6[a]	22.1±23.6[b]	21.9±19.5[c]	21.8±18.5[d]
	1T 25.2±2.15[a]	24.9±2.26[b]	24.2±3.56[c]	22.4±2.66[d]
	2T 34.2±3.26[a]	29.3±2.16[b]	28.8±2.91[c]	29.0±2.96[d]
	3T 27.3±1.92[a]	27.1±1.95[b]	26.3±1.79[c]	24.1±2.51[d]
UA(µmol.L-[1])	SD 219.8±7.33[a]	220.2±9.41[b]	218.9±8.32[c]	219.6±7.91[d]
	1T 283.1±22.6[a]	268.1±19.4[b]	166.1±17.3[c]	77.1±9.15[d]
	2T 300.1±19.2[a]	218.2±18.6[b]	176.1±15.1[c]	89.0±5.12[d]
	3T 274.1±16.4[a]	257.8±19.1[b]	158.2±13.9[c]	68.0±3.65[d]

SD standard diet, 1T diet with single strength *Arthrospira*, 2T standard diet with twice strength *Arthrospira*, 3T standard diet with trice strength *Arthrospira*; Different superscripts for the same variable mean implied statistical differences ($p<0.05$), N number of animals in the group=4.

Table 5 showed the increase in the values of chicken total protein (TP), albumin and total biliprotein while GGT remained stable for the treated groups during the 40 day feed trial study period. The change in the level of total protein (TP) and albumin as well as the total biliprotein (TB) level to a large extent, had significant influence the chicken blood chemistry. There was a significant increase in the blood protein levels in the *Arthrospira* treated groups with values of 16% (initial value 50.0±5.21 g.L^{-1}) and 15% (initial value 52.8±6.16 g.L^{-1}) increment on day 40 for the 1T and 3T groups respectively; however the SD group did not showed any significant change or variation. In the case of albumin content, there was a slight increase in the blood albumin value from 24 to 31% of their initial values in *Arthrospira* supplement fed chicken; while that of the total biliprotein showed an increase with values ranging from 17 to 28% of their initial values. For the SD diet group, there was a decrease of total protein from 54.0±3.05g.L^{-1} to 48.4±3.42g.L^{-1} indicating a 11.1% drop in the protein.

Table 5: Values of total protein (TP), albumin (Alb), total biliprotein (TB), and gamma glutamyl transaminase (GGT) in chicken for 40 day of treatment with diets (SD, T, 2T and 3T).

Time (d)	10	20	30	40
TP (g.L-[1])	SD 54.0±4.06[a]	51.3±3.67[b]	49.1±3.11[c]	5.61±0.36c
	1T 50.0±5.21[a]	52.0±4.17[b]	55.0±3.64[c]	58.0±7.12[d]
	2T 55.0±5.43[a]	58.8±0.06[b]	60.1±0.06[c]	65.1±0.06[d]
	3T 52.8±6.16[a]	53.1±5.33[b]	56.1±3.63[c]	60.8±7.13[d]
Albumin (g.L-[1])	SD 17.1±2.16[a]	17.2±2.06[b]	17.9±1.81[c]	17.0±2.11[d]
	1T 16.1±1.72[a]	16.3±1.91[b]	16.5±2.15[c]	20.1±1.91[d]
	2T 14.1±1.91[a]	15.0±1.34[b]	17.8±1.62[c]	19.1±1.94[d]
	3T 16.2±1.51[a]	17.1±1.81[b]	18.6±1.16[c]	20.1±1.07[d]
TB (mg. L-[1])	SD 1.70±0.16[a]	1.78±0.12[b]	1.79±0.28[c]	1.77±0.13[d]
	1T 2.30±0.13[a]	2.28±0.16[b]	2.31±0.21[c]	2.70±0.42[d]
	2T 3.41±0.16[a]	2.97±0.17[b]	2.81±0.21[c]	2.70±0.37[d]
	3T 2.11±0.26[a]	2.32±0.16[b]	2.41±0.26[c]	2.71±0.43[d]
GGT (U.L SD)	24.6±2.17[a]	24.7±2.26[b]	24.5±2.12[c]	24.7±1.89[d]
	1T 24.4±2.21[a]	24.4±2.51[b]	24.5±2.46[c]	24.3±3.15[d]
	2T 19.7±1.42[a]	19.2±1.36[b]	19.3±2.13[c]	19.6±1.51[d]
	3T 23.8±1.88[a]	23.7±2.29[bc]	23.6±1.91[c]	23.8±1.60[c]

SD standard diet, 1T diet with single strength *Arthrospira*, 2T standard diet with twice strength *Arthrospira*, 3T standard diet with trice strength *Arthrospira*; Different superscripts for the same variable mean implied statistical differences ($p<0.05$), N number of animals in the group=4.

The requirement of glycine and serine are metabolically interconvertible and their inclusion in the feed diet are of utmost important (Akrabawi & Kratzer, 1968). Improvements in the growth rate and efficiency of feed utilization were observed when the diet was supplemented with 0.2 to 0.4% L-proline (Graber & Baker, 1973). Chickens are sensitive to the dietary balance of amino acids involving imbalances and antagonisms among the amino acids (Harper et al., 1970). When using the ideal amino acid ratio concept, the digestible rather than total amino acid contents of feed ingradients must be considered in formulating feeds (Emmert & baker1997; Baker et al. 2002). The three branched chain amino acids in *Arthrospira* sp viz. leucine, valine and isoleucine (Gunsett, 1984) are responsible for the increase in body weight in farmed poultries or fish (Rodehutscord et al., 1997; Ahmed & Khan, 2006; Peganova & Eder, 1997, Gershwin & Belay, 2008). Furthermore, the GGT levels also did not showed any noticeable change in the present experimental groups (SD, 1T, 2T, 3T) during the study period.

Interestingly, in the present experiment, the level of blood calcium did increase slightly with values of 1.4% (initial value 5.50±0.32 mmol.L^{-1}) and 4.8% (initial value 5.60±0.31 mmol.L^{-1}) on day 40 for the 1T and 3T groups respectively; however the SD group did not showed any significant change (Table 6). It is noted in for both groups, the percentage increase in blood calcium is insignificant with increase *Arthrospira* supplement. Similar trend is observed in the blood K content for the treated groups with an increment value of 2 to 3%. Moreover, the high cyanobactera protein supplement increased significantly the AST level with the value of 17.3% (initial value 149.8±12.5 U.L^{-1}) and 29.2% (initial value 130±11.2U.L^{-1}) on day 40 in the treated T and 3T groups respectively while the SD group has its AST level being stabilized throughout the experimental period. ALT values obtained in the treated groups (T, 2T, and 3T) are within the chicken normal range (6.0-7.5U.L^{-1}); however, the ALT values increased significantly from 1.7 to 4.8% of their initial values. As a comparison to chicken, the experiment carried out by El-Sabagh *et al*., (2014) on young lambs resulted in the drop of AST level by 13%. In this present experiment, the ALT and AST levels in the SD group showed very little variation in their blood values during the study period.

Table 6: Values of AST, ALT, Calcium (Ca) and Potassium (K) in chicken for 40 day of treatment with diets (SD, T, 2T and 3T).

Time (d)		7	14	21	28
AST (U. L-^{1})	SD		180.0±16.6^{a}	178.2±15.1^{b}	178.2±16.6^{c}
180.2±16.6^{d}	1T	150.0±14.6^{a}	169.2±17.6^{b}	171.2±15.6^{c}	176.3±11.3^{d}
	2T	186.0±18.6^{a}	188.8±15.2^{b}	210.1±19.3^{c}	225.1±21.2^{d}
	3T	130.0±11.2^{a}	136.1±10.6^{b}	158.1±14.5^{c}	168.8±17.1^{d}
ALT (U. L-^{1})	SD		6.98±0.71^{a}	7.01±0.91^{b}	6.97±0.94^{c}
6.98±0.95^{d}	1T	6.95±0.34^{a}	6.93±0.41^{b}	6.89±0.21^{c}	7.07±0.21^{d}
	2T	6.11±0.34^{a}	6.04±0.54^{b}	5.99±0.64^{c}	5.97±0.43^{d}
	3T	7.15±0.51^{a}	7.11±0.59^{b}	6.92±0.56^{c}	6.80±0.61^{d}
Ca(mmol. L-^{1})	SD	5.80±0.21^{a}	5.78±0.19^{b}	5.79±0.32^{c}	5.77±0.19^{d}
	1T	5.50±0.32^{a}	5.58±0.17^{b}	5.51±0.27^{c}	5.58±0.17^{d}
	2T	5.25±0.29^{a}	5.17±0.28^{b}	5.30±0.21^{c}	5.41±0.21^{d}
	3T	5.61±0.31^{a}	5.70±0.21^{b}	5.71±0.28^{c}	5.88±0.27^{d}
K(mmol. L-^{1})	SD		5.79±0.71^{a}	5.75±0.77^{b}	5.81±0.96^{c}
5.78±0.77^{d}	1T	5.40±0.61^{a}	5.42±0.37^{b}	5.51±0.31^{c}	5.50±0.51^{d}
	2T	5.60±0.31^{a}	5.58±0.29^{b}	5.61±0.27^{c}	5.71±0.45^{d}
	3T	5.51±0.30^{a}	5.58±0.32^{b}	5.61±0.36^{c}	5.68±0.21^{d}

SD standard diet, 1T diet with single strength *Arthrospira*, 2T standard diet with twice strength *Arthrospira*, 3T standard diet with trice strength *Arthrospira*; Different superscripts for the same variable mean implied statistical differences ($p<0.05$), N number of animals in the group=4.

The AST, ALT and GGT of the hepatic serum function also specialized in signaling the problems of the liver whereby these enzymes are released into the blood as a result of cellular impairment or injury. ALT served as the most specific indicator of hepatic injury; however GGT functions as a sensitive marker but not as specific as ALT (Al-Sultan, 2008). Studies on the effect of high protein supplement on chicken are scare and rare; thus, comparison has to be made on other animals. Fish are among the most efficient of animals in converting food energy to body tissue, partly because they require less energy for maintenance required by birds or mammals of the same size. As a consequence, protein efficiencies (protein gain/ protein fed) of 45% have been seen in catfish fed diets containing 11.4 Kcal of digestible energy (DE) per gram of protein (Gatlin *et al*., 1986). Optimum DE to protein ratios that have been determined for fish are twice dietary protein for terrestrial farm animals (NRC, 1993).

For Ca and P fish uptake requirement, the availability of these minerals in food varies with their numerous forms and structures, amounts of other interacting elements as well as occurring in various species of poultry. As a comparative to chicken requirement of phosphorus in the aqua-livestock feed meal is higher for rainbow trout and chum salmon than for tilapia and common carp, because of the limited secretion of gastric juices by the latter warm-water species (Ogino et al., 1970; Yone & Toshima, 1979). In the case of calcium uptake from seawater is sufficient for Atlantic salmon (Lall & Bishop, 1977) but insufficient for red seabream, which still require 0.34% calcium in the air dry diet (Sakamoto & Yone, 1976). Dietary sources of minerals can differ in biologically available mineral content; P in dicalcium phosphate has greater availability than P in raw phosphate (Scott et al.,1982; Pond et al., 2005). The phosphate in phytin can be released by intestinal phytase, but the activity of this enzyme is limited only in nonruminant such as poultry. Phosphorus availability can be increased by the addition of phytase to diets (Pond et al., 2005). For feed formulation,it is assumed that the P in feedstuffs of plant origin is 30% available (Scott et al., 1982).

4. CONCLUSION

Until this present time, studies on the effectiveness of the *Arthrospira* supplement protein meal and pre-clinical practice on the local chickens seem to be scarce. This also reflects the ability of the high protein meal supplement to function as an anti-hypercholesterolemic agent in local chickens. It also provided the betterment in the chicken health, blood chemistry and growth performance. The protein supplement had increased the HDL level and lowered the cholesterol as well as stabilized the GGT blood level without indicating any adverse effects towards kidney-liver function. As a consequence, the protein supplement production and requisition have been on the increase since the proliferation of its usage as supplement products which are free from toxic chemicals. In view of the facts that the present chicken feeds available locally or imported from abroad have been dreadfully adulterated with toxic growth promoters such as hormones and antibiotics, we need to replace or supplement the existing feeds with safe, natural and healthy feed derived especially from locally plant, animal or cyanobacteria materials. In the case of protein supplement meal of local source should contain beneficial materials such as, protein, essential amino acids, polyunsaturated fatty acids, vitamin and mineral contents. Using the local chicken as a model test animal, the present study showed the usage of this supplement diet inclusion at the recommended level for the chicken meal can provide the positive effects on the growth, feed conversion and plasma lipoprotein lipase activity in chicken.

ACKNOWLEDGEMENTS

Authors would like to express their thank to Malaysian Ministry of Higher Education for the grant (R/PRGS/ B01.08/01580A/001/2016/000384) awarded and Kelantan State Veterinary Services Department for assisting this project.

REFERENCES

1. Ahmed, I., & Khan, M.A. Dietary branched chain amino acid valine, isoleucine and leucine requirements of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *British Journal of Nutrition*, 2006; 96: 450-460.
2. Allard, J.P., Royall, D., Kurian, R., Muggli, R., & Jeejeebhoy, K.N. Effects of beta-carotene supplementation on lipid peroxidation in humans. American Journal of Clinical Nutrition, 1994; 59(4): 884-890.
3. AOAC. Official methods of analysis of AOAC International. 16th ed. Arlington: Virginia AOAC International, 1997.
4. Almendingen, K., Jordal, O., Kierulf, P., Sanstad, B., & Pedersen, J.I. Effects of partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp(a) in men. *Journal of Lipid Research*, 1995; 36(6): 1370-1384.
5. Al-Sultan, A.I. Assessment of the relationship of hepatic enzymes with obesity and insulin resistance in adults in Saudi Arabia. *Sultan Qaboos University Medical Journal*, 2008; 8(2): 185-192.
6. Bhowmik, D., Dubey, J., & Mehra, S. Probiotic efficiency of Spirulina platensis-stimulating growth of lactic acid bacteria. World Journal of Dairy and Food Science, 2009; 4(2): 160-163.
7. Belay, A. The potential application of Spirulina as a nutritional health and therapeutic supplement in health management. Journal of the American Nutraceutic Association, 2002; 5(2): 27-48.
8. Bonos, E., Kasapidou, E., Kargopoulos, A., Karampampas, A., Christaki, E., Florou-Paneri, P., & Nikolakakis, I. Spirulina as a functional ingredient in broiler chicken diets. South African Journal of Animal Science, 2016; 46(1): 94-101.
9. Calder, P.C. n-3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clinical Science, 2004; 107: 1-11.
10. Carpenter, K.L., Kirkpatrick, P.J., Weissberg, P.L., Challis, I.F., Freeman, M.A. & Mitchinson, M.J. Oral alpha-tocopherol supplementation inhibits lipid oxidation in established human atherosclerotic lesions. Free Radical Research, 2003; 7: 1235-1244.
11. Carty, J.L., Bevan, R., Waller, H., Mistry, N., Cooke, M., Lunec, J. & Griffiths, H.R. The effects of vitamin C supplementation on protein oxidation in healthy volunteers. Biochemical Research Communications, 2000; 273: 729-735.
12. Coche, A.G. & Muir, J.F. Management for fresh water fish culture, fish stocks and farm management. Rome: Food and Agriculture Organization of the United Nations, 1998.
13. Colla, L.M., Muccillo-Baisch, L. & Costa,J.A.A. Spirulina platensis effects on the levels of total cholesterol, HDL and triglycerols in rabbits fed with a hypercholsterolemic diet. Brazillian Archieves of

Biology and Technology, 2008; 51(2): 405-411.
14. Daghir, N.J. Poultry Production in Hot Climates, 2nd Ed. CAB International, Wallinford, Oxfordshire, UK, 2008.
15. Dauber, D., Horlich, L., & Katz, L.N. The role of dessiated thyroid and potassium iodide in the cholesterol-induces atherosclerosis of the chicken. American heart Journal, 1949; 38: 25-33.
16. Dauber, D.V. Spontaneous atherosclerosis in chickens. American Medical association Archieves of Pathology, 1944; 38: 46-51.
17. Deng, R. and Chow, T.J. Hypolipidemic, antioxidant and anti-inflammatory activities of Spirulina, Cardiovascular Therapeutics, 2010; 28(4): 33-45.
18. Devi, M.A. & Venkataraman, L.V. Hypocholestemic effect of bluegreen algae Spirulina platensis in albino rats. *Nutrition Reports International*, 1983; 28: 519-530.
19. Edjeng Suprijatna. Siri Pertanian dan Industri Kecil; Perusahan Ayam Kampung Sebagai Ayam Telur. Edisi kedua, Mardi, 2008; 153.
20. El-Sabagh, M.R., Eldaim, M.A.A., Mahboub, D.H. & Abdel-Daim, M. Effects of Spirulina platensis algae on growth performance, antioxidative status and blood metabolites in fattening lambs. *Journal of Agricultural Sciences*, 2014; 6(3): 92-96.
21. Engel, S., & Tholstrup, T. Butter increased total and LDL cholesterol compared with olive oil resulted in higher HDL cholesterol compared with a habitual diet. *American Journal of Clinical Nutrition*, 2015; 102(2): 309-315.
22. Gatlin D.M.,III, Poe W.E. & Wilson R.P. Protein and energy requirements of fingerling channel catfish for maintenance and growth. *J. Nutr*, 1986; 116: 2121-2131.
23. Gershwin, M.E. & Belay, A. Spirulina in human nutrition and health. London: Taylor & Francis Ltd, 2008.
24. Gunsett, F.C. Linear index selection to improve traits defined as ratios. *Journal of Animal Science*, 1984; 59: 1185-1193
25. Heidarpour, A., Fourouzandeh-Shahraki, A.D. & Eghbalsaied, S. Effects of Spirulina platensis on performance, digestibility and serum biochemical parameters of Holstein calves. *African Journal of Agricultural Research*, 2011; 6(22): 5061-5065.
26. Iwata, K. Inayama, T., & kato, T. Effects of Spirulina platensis on plasma lipoprotein lipase activity in rats. *Journal Nutrition Science Vitaminology*, 1990; 36, 165-171.
27. Jaime-Ceballos, B., Villareal, B.H., Garcia, T., Perez J.A.R., & Alfonso, E. 2005. Effect of Spirulina platensis meal as feed additive on growth, survival and development in Litopenaeus schmitti shrimp larvae, Rev. Invest. Mar. 26: 235-241.
28. Kamalaveni, Santhosh, B., & Sithara, K. Evaluation of Spirulina supplemented diet and its influence on bioenergetics and biochemical parameters in the fish *Cirhinus mrigala*. Nature Environment and Pollution Technology, 2009; 8(2): 351-354.
29. Kaoud, H. Effect of *Spirulina platensis* as a dietary supplement on broiler performance in comparison with prebiotics. Scientific Journal of Applied research, 2012; 1(2): 44-48.
30. Kharde, S.D., Shirbhate, R.N., Bahiram, K.B. & Nipane, S.F. Effect of *Spirulina* supplementation on growth performance of broilers Indian Journal of Veterinary Research, 2012; 21: 66-69.
31. Kim, M.H. & Kim, W.Y. The change of lipid metabolism and immune function caused by antioxidant material in hypercholesterolemia elderly women in Korea. Korean Journal of Nutrition, 2005; 38: 67-75.
32. Kolanowski, W., Swiderski, F. & Berger, S. Possibilities of fish oil application for food porducts enrichment with omega-3 PUFA. International Journal of Food Sciences and Nutritionn, 1999; 50: 39-49.
33. Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. Fish consumption, fish oil, omega-3 fatty acids & cardiovascular diseases. Circulation, 2002; 106: 2747-2757.
34. Lall, S.P., & Bishop, F.J. Studies on mineral and protein utilization by Atlantic salmon grown in sea water. *Fish. Mar. Serv. Tech. Rep*, 1977; 688: 1-16.
35. Lokman-Shamsudin, Syarifah AR, Azman N.A., Wan Zahari M. & Hafis H.L. The milk index, blood chemistry status and growth performance of local Malaysian cow (*Bos Sundoicus*) fed *Arthrospira platensis* supplement. *Malays. Appl. Biol.,* 2018; 47(4): 1-9.
36. Mani, U.V., Desai, S. & Iyer, U. Studies on the long term effect of Spirulina supplementation on serum lipid profile and glycated proteins in NIDDM patients. Journal of Nutraaceuticals, Functional and Medical Foods, 2002; 2(3): 25-32.
37. Mariey, Y.A., Samak, H.R., & Ibrahem, M.A. Effect of using *Spirulina platensis* alga as a feed additive for poultry diets: 1- Productive and reproductive performance of local laying hens. Egyptian poultry Science Journal, 2014; 32: 201-215.
38. Masegi,T., Sato, K., Kawada, M., Yanai, T., & Ueda, K. Spontaneous coronary artheriolosclrosis in broiler chickens. Journal of Veterinary Medical Science, 1993; 55: 457-459.
39. McKee, J.S., Harrison, P.C., & Riskowski, G.L., Effects of supplemental ascorbic acid on the energy conversion of broiler chicks during heat stress and feed withdrawal. Poultry Science, 1997; 76: 1278-1286.
40. Muhammad Nazrul, S., Nor Azura M., Zuraini, A., Zainul, A., Lokman-Shamsudin, Sofian, M., & Arifah, A.K. Anti-inflammatory and anti-pyretic properties of *Spirulina platensis* and *S. lonar* A comparative study, Pakistan Journal of Pharmaceutical Sciences, 2014; 27(5): 1277-1280.
41. North, M.O., & Bell, D.D. Commercial chicken production manual, 4th ed. Van Nostrand Reinhold, New York, NY, 1990; 262.
42. Nagaoka, S., Shimizu, K., Kaneko, H., Shibayana,

F., Hirahashi, T. & Kato, T. A novel protein c-phycocyann plays a crucial role in the hypocholesterolemic action of *Spirulina platensis* concentrate in rats. Journal of Nutrition, 2005; 135(10): 2425-2430.
43. National Research Council (NRC). *Nutritient Requirements of Fish.* National Academy Press, Washington, DC, 1993.
44. National Research Council. Subcommittee on Warmwater Fishes. Nutrient requirement. National Academy of Sciences, Washington, D.C., 1977; 78
45. National research Council (NRC) Nutrient Requireents of Poultry, 9th rev. ed. National Academy Press, Washington, DC, 1994.
46. Ogino, C., Takeuchi, L., Takeda, H., & Watanabe, T. Availability of dietary phosphorus in carp and rainbow trout. Bull. Jpn. Soc. Sci. Fish, 1979; 45: 1527-1532.
47. Orita, S., Maegi, T., Itou, K., Kawada, M., Yanai, T. & Ueda, K. Spontaneous aorta arteriosclerosis in layer chickens, Journal of Comparative Pathology, 1994; 110: 341-347.
48. Otles, S. & Pire, R. Fatty acid composition of *Chlorella* and *Spirulina* microalga species. Journal of AOAC International, 2001; 84(6): 1708-1714.
49. Patterson, E., Wall, R., Fitzgerald, G.F., Ross, R.P. & Stanton, C. Health implications of High Dietary Omega-6 Polyunsaturated Fatty Acids. Journal of Nutrition and Metabolism, 2012; 53-94.
50. Page J.W. & Andrews. Interactions of dietary levels of protein and energy on channel catfish *(Ictalurus punctatus). J. Nutr,* 1973; 103: 1339-1346.
51. Peganova S. & Eder K. Interactions of various supplies of isoleucine, valine, leucine and tryptophan on the performance of laying hens. *Poultry Science*, 2003; 82: 100-105.
52. Pond, W.G. Church, D.C., Pond, K.R. & Schoknecht, P.A. Basic Animal Nutrition and Feeding, Wiley, 2005; 479.
53. Pond, W.G., Church, D.C., Pond, K.R., & Schoknecht, P.A. 2005. Basic Animal Nutrition and Feeding, 5th Ed. Wiley, 479.
54. Rodehutscord M., Becker A., Pack M. & Pfeffer E. Response of rainbow trout (*Oncorhynchus mykiss*) to supplements of individual essential amino acids in a semi purified diet, including an estimate of the maintenance requirement for essential amino acids. *Journal of Nutrition*, 1997; 127(6): 1166-1175.
55. Sakamoto, S., & Yone, Y. Requirement of red sea bream for dietary Ca. Rep. Fish. Res. Lab. Kyushu Univ, 1976; 3: 59-64.
56. Salehi-Farsani, A., Soltani, M., Kamali, A. & Shamsaie, M. Effect of immune motivator macrogard and *Spirulina platensis* on some growth, carcass and biochemical indices of stellate sturgeon Acipenser stellatus. AACL Bioflux, 2014; 7(3): 137-147.
57. Schwartz, M., Schultz, Y., Piolino, V., Schneider, H. & Febler, J.P. Thermogenesis in obese women: effect of fructose vs. glucose added to a meal. American Journal of Physiology, 1992; 262: 394-401.
58. Scott, M.L., Nesheim, M.C., & Young, R.J. *Nutrition of the Chicken.* M.L. scott & Associates, Ithaca, NY, 1982.
59. Shamekh, R., Linden, E.H., Newcomb, J.D., Tigno, X.T., Jen,Pellizzon, M.A. & Hansen, B.C. Endogenous and diet-induced hypercholesterolemia in nonhuman primates: effects hypercholesterolemia of age, adiposity and diabetes on lipoprotein profiles. Metabolism, 2011; 60(8): 1165-1177.
60. Shanmugapriya, B., & Saravana, B.S., Supplementary effect of *Spirulina platensis* on performance, hematology, and carcass yield of broiler chicken. Indian streams research Jurnal, 2014; 4: 1-7.
61. Shih, J.C.H., Pullman, E.P. & Kao, K.J. Genetic selection, general characterization, and histology of atherosclerosis susceptible and resistant Japenese quail. Atherosclerosis, 1983; 49: 41-53.
62. Simopoulos, A.P. Essential fatty acids in health and chronic disease. American Journal of Clinical nutrition, 1999; 70(3): 560-569.
63. Sinn, N., Milte, C.M., Street, S.J., Buckley, J.D., Coates, A.M., Petkov, J. & Howe, P.R.C. Effects of a n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: a 6-month randomized controlled trial. British Journal of Nutrition, 2012; 107: 1682-1693.
64. Snowmya A., & Ananthi, T. Hypolipidemic activity of *Mimosa pudica* Linn on butter induced hyperlipidemia in rats. *Asian Journal of Pharmaceutical Sciences*, 2011; 1(4): 124-127.
65. Swinburn B.A., catersin I., Seidell J.C. & James W.P.T. Diet, nutrition and the prevention of excess weight gain and obesity. *Public Health Nutrition*, 2004; 7(1A): 123-146.
66. Takayama M, Itoh H, Nagasaki T, & Tanimizu, I A new enzymatic method for the determination of serum choline containing phospholipids, *Clin. Chim. Acta*, 1977; 79: 93-98.
67. Tovar, D., Zambononino, J., Cahu, C., Gatesoupe, F.J., Vazquez, J.R. & Lesel, R. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass larvae. Aquaculture, 2002; 204: 113-123.
68. Tuttle, W.L. & Balloun, S.L. Leucine, Isoleucine and Valine Interactions in Turkey Poults. Poutry Science, 1976; 55: 1737-1743.
69. Yone, Y., & Toshima, N., The utilization of phosphorus in fish meal by carp and black sea bream. *Bull. Jpn. Soc. Sci. Fish*, 1979; 45: 753-756.
70. Walton, M.J., Coloso, R.M., Cowey, C.B., Adron, J.W., & Knox, D. The effects of dietary tryptophan levels on growth and metabolism of rainbow trout *Salmo gairdneri.* Br. J. Nutr, 1984; 51: 279-287.
71. Wardlaw G.M. & Snook J.T. Effect of diets high in butter, corn oil, or high oleic acid sunflower oil on serum lipids and apolipoproteins in men. *American*

Journal of Clinical Nutrition, 1990; 51: 815-821.
72. Scott, M.L., Nesheim, M.C., & Young, R.J. *Nutrition of the Chicken.* M.L. scott & Associates, Ithaca, NY, 1982.
73. Weaver, K.L., Ivester, P., Chilton, J.A., Wilson, M.D., Pandey, P. & Chilton, F.H. The content of favorable and unfavourable polyunsaturated fatty acids found in commonly eaten fish. Journal of the American Dietetic association, 2008; 108(7): 1178-1185.
74. Wilson, R.P. & Halver J.E. Protein and amino acid requirements of fishes. *Annu. Rev. Nutr,* 1986; 6: 225-244.
75. Wolfenson, D., Frei, Y.F., snapir, N. & Berman, A. Heat stress effects on capillary blood flow and its retribution in the laying hen. Pflugers Archiv, 1981; 390(1): 86-93.
76. Xiangdong, L., Yuanwu, L., Hua, Z., Liming, R., Qiuyan, L. & Ning, L. Animal models for the artgerosclerosis research: a review. Protein and Cell, 2011; 2(3): 189-201.
77. Yin,W., Carballo-Jane,E., McLaren, D.G., Mendoza, V.H., Gagen, K., & Geoghagen, N.S. Plasma lipid profiling across species for the identification of optimal models of human dyslipidemia. Journal of Lipid Research, 2012; 53(1): 51-65.
78. Zare, K., Tabatabael, S.R.F., Shahriari, A. 7 Jafari, R.A. The effect of butter oil on avoidance memory I normal and diabetic rats. Iranian Journal of Basic Medical Sciences, 2012; 15(4): 983-989.